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Fatty Degeneration, Regenerative Hyperplasia and Neoplasia in the Livers of Rainbow Trout, *Salmo gairdneri*

Ross F. Nigrelli & Sophie Jakowska
New York Aquarium, New York Zoological Society

(Plates I-VI)

Introduction

LIVER tumors in fish were first reported in European brown trout (*Salmo trutta* Linnaeus) by Plehn (1909a, 1924). The first case in rainbow trout (*Salmo gairdneri* Richardson) was described by Haddow & Blake (1933) in England. The disease was also reported in this species in the United States in 1953 by Nigrelli (1954), who indicated that the incidence of hepatomas in such trout might be relatively high. Four tumors were subsequently found in a single lot of 600 3-year-old rainbow trout in a Pennsylvania hatchery, and the histopathology of these was briefly described by Nigrelli & Jakowska (1955). Almost simultaneously, similar tumors that had been reported by Scolari in 1953 in rainbow trout from all the hatcheries along the Alpine Arc and the lake region of northern Italy, were described by Cudkowicz & Scolari (1955). They reported an incidence of 50% or more in some hatcheries and described the histopathology and distribution of the disease and indicated its possible cause.

In the spring of 1960, rainbow trout that were to be introduced into California's waters were examined at the state line by California Fish and Game inspectors and found to be affected with liver tumors. An embargo was immediately placed on all rainbow trout being shipped into the state. This led to the examination of trout in many hatcheries, whereupon it soon became evident that trout with liver diseases were widely distributed in the United States, with an incidence of hepatomas of 50% or more being reported in some hatcheries. In retrospect, some fishery personnel have indicated that the hepatomas were prevalent in rainbow trout as far back as 1937 (Rucker *et al.*, 1961). It is highly probable that the disease went unrecognized for a great number of years, since little or no attention was given to fish pathology by fishery biologists in this country until relatively recently.

Since no critical distinction has been made between regenerative hyperplasia and neoplasia, we cannot accept the numerous unofficial reports that all the "tumors" seen in rainbow trout are hepatomas, especially if the term is used to indicate a neoplastic process. The present paper is therefore principally concerned with a microscopic analysis of the livers of rainbow trout from several hatcheries, mainly in Idaho, Montana and Wyoming, and those of a few specimens of brown and rainbow trout taken from natural waters.

Observations and Discussion

Three hundred and thirty-three livers were examined. They varied considerably in size and appearance, and those with apparent lesions were characterized by grayish or yellowish spots, streaks (Plate I, Fig. 1), numerous petichiae or larger clots, or by one or more encapsulated (Plate I, Fig. 2) and non-encapsulated nodules on the surface or deep in the parenchyma. In a few instances, livers with hob-nail or miliary appearance were also seen.

The so-called normal liver showed a histological picture resembling that consistently found in teleosts kept in captivity and fed artificial diets. The usual pattern consisted of lobules with a central vein in which radiating liver cells, separated by sinusoids, were arranged as a muralium[1], or wall, two cells in thickness but without polar orientation of the nucleus (Plate

[1] The significance of two-cell-thick muralia in hepatomas of mammals and birds is discussed by Elias (1955).

III, Fig. 6). The over-all picture was one of uneven staining, suggesting physiological differences in the cells of the various lobules. The liver cells were uniform in size and typical in shape, but some relatively larger cells with larger nuclei, suggestive of polyploidy, were also observed. The cytoplasm was usually lightly basophilic, but the degree of basophilia sometimes varied with the lobules, apparently affected by the presence or absence of cytoplasmic particulates. The nucleus was typical, with one or more acidophilic nucleoli. Although binucleate and multinucleate cells were not unusual, mitotic figures were rare.

After appropriate staining, changes indicative of disease were interpreted as fatty infiltration (Plate II, Fig. 3), glycogen infiltration or depletion, ceroid deposition (Plate II, Fig. 4), hematochromatosis (Plate II, Fig. 5), focal necrosis with lymphocytic infiltration (Plate III, Figs. 6, 7), portal cirrhosis (Plate III, Fig. 8), biliary cirrhosis (Plate III, Fig. 9), hyperplasia and neoplasia.

Hyperplasia, which ranged from simple solitary (Plate IV, Fig. 10) or widely diffuse regenerative areas (Plate IV, Fig. 12) to single or multiple nodules, was found in 50 of the livers. The hyperplasia was evident as islands or as extensive growth of liver cells of irregular pattern in which uninucleate and multinucleate elements were densely packed, especially at the periphery of the nodules. Some of the larger nodules were completely necrotic. In some areas, clusters of larger cells with acidophilic cytoplasm and eccentric nuclei were present (Plate IV, Fig. 11). The origin and nature of these cells, some of which contained ceroid, remain to be determined. In close proximity to these, as well as in areas adjacent to regions of apparent active growth, there were considerable morphological changes in the liver cells, and these changes extended even to elements more distantly located. The lobules were often distorted and the cells showed variation in size, shape and staining reactions; in many instances the tissue assumed an adenomatoid appearance (Plate V, Fig. 13). These effects were more pronounced in hyperplastic livers with extensive fatty infiltration and ceroid deposition and with portal and biliary cirrhosis.

Nineteen of the livers were diagnosed as neoplastic and the changes noted in them ranged from adenoma (Plate IV, Fig. 14) to hepatocellular carcinoma. The latter exhibited either a normal cell arrangement (but without distinct lobular pattern), or an anaplastic, highly pleomorphic structure with considerable degeneration and hemorrhage. The hepatocellular lesions were frequently indicated by a solid cord-like cellular arrangement in which the hepatic cells were relatively small, the nuclei pyknotic and vascularization scanty (Plate V, Fig. 15). Cholangiomatous changes (Plate VI, Fig. 16) and cirrhosis (Plate VI, Fig. 17) were found in a few cases. Other details were similar to those reported by Nigrelli & Jakowska (1955) and by Cudkowicz & Scolari (1955).

Some of the abnormal conditions described above, including adenomatous and cholangiomatous growths, were also found in livers of four rainbow trout and Loch Leven (brown) trout taken from natural waters in Idaho.

The histological picture of the atypical cell growths in the livers of hatchery-raised rainbow trout is strikingly similar to that reported in mice and other experimental animals kept for prolonged periods on a low choline diet, and in which fatty livers with little or no cirrhosis develop (Hartroft, 1954, 1955). In general, liver diseases in higher animals involving necrosis, fatty changes and ceroid deposition, with or without hyperplasia or neoplasia, have been attributed to deficiency or imbalance of one or more of the following substances: methionine, cystine, choline, vitamin E, vitamin B_{12} and riboflavin (Kensler *et al.*, 1941; Endicott & Lillie, 1944; Engle *et al.*, 1947; Lee, 1950; Casselmen, 1953; Daft, 1954; Drill, 1954; Schwarz, 1954; Hartroft, 1954, 1955; Salmon & Copeland, 1954; Salmon *et al.*, 1955; see also Tannenbaum, 1953).

Although most of the above essential substances are incorporated into artificial trout diets in the United States, it is generally agreed that the complete nutritional requirements of trout have not yet been fully determined, a fact which is in part responsible for the wide variety of diets still in use in hatcheries here and abroad. The non-tumor and regenerative lesions described in this paper may be indicative of some nutritional faults. Some of these abnormal conditions, especially in relation to fatty degeneration and ceroid deposition, have been previously reported in experimentally- and non-experimentally-fed hatchery trout (Plehn, 1909b, 1915, 1924; Gaschott, 1929; Hewitt, 1937; McLaren *et al.*, 1946 ;Mann 1952; Davis, 1953; Schäperclaus, 1954; Scolari, 1954; Faktorovich, 1956a,b, 1958, 1960). Some striking effects of purified rations on the livers of rainbow trout were shown by McLaren *et al.* (1946). Yearling rainbow trout, fed a ration composed of cerelose 48%, casein 40%, fat 2%, dried liver 5% supplemented with brewers' yeast, were able to maintain, for a period of time, a hemoglobin level and growth rate equal to that produced by feeding 100% dried liver or a standard hatchery

meat ration. After 8 weeks, however, the fish suddenly died and autopsies showed that they had developed greatly enlarged, yellow, lobulated livers. In this case, the liver damage was prevented by reducing the carbohydrate level to 20%.

The most important disease in hatchery-raised rainbow trout is generally considered to be fatty degeneration of the liver, but with proper diet recovery is often complete. Thus, Faktorovich (1956b) for 11 months carefully followed the regeneration of hepatic tissue in rainbow trout during recovery from fatty degeneration, a condition which he later (1960) interpreted as taking the form of ceroid deposition. It should be emphasized, however, that ceroid deposition appears to be a general characteristic of teleosts, especially those kept in captivity (Pickford, 1953; Wood & Yasutake, 1956; Nigrelli, 1960), those infected with parasites (Nigrelli, 1954), or those exposed to toxic substances, *e.g.*, copper (Calventi *et al.*, 1960).

The epizootic nature of the liver diseases in rainbow trout suggests that hereditary, viral or carcinogenic agents may also play a role. The possibility that the tumors are hereditary in origin has been indicated by Cudkowicz & Scolari (1955). Evidence for this suggestion is based on the following: (1) rainbow trout in the hatcheries in northern Italy are all the progeny of fish imported from Rocky Mountain streams in 1880, (2) a high incidence of tumors was found in populations derived from a stock inbred for 20 years in one hatchery, (3) the liver tumors were not found in hatcheries in Switzerland and Bavaria, two areas that had had no exchange of fish with the Italian hatcheries, and (4), rainbow trout from Denmark that were raised in the Italian hatcheries under identical conditions were not affected with the growths.[2]

Rainbow trout in the United States have been artificially bred for approximately the same length of time as in Europe. Although inbreeding records are not available to us, hatchery practices, such as discarding runts and selecting highly colored or early maturing fish, inevitably lead to the selection of special strains—possibly with inherent susceptibility to liver dysfunctions, *e.g.*, inability to metabolize fats properly. Whether or not such strains can be associated with specific genetic factors remains to be determined. In certain mouse colonies, mutant strains (CBA and C3H) highly susceptible to hepatomas have spontaneously appeared (Andervont, 1950; Woolley, 1951).

Virus and carcinogens as etiological agents cannot be excluded, but mammal geneticists generally agree that their principal effect is to enhance the existing intrinsic susceptibility to spontaneous tumors (Heston, 1941; Heston & Deringer, 1947, 1949; Duran-Reynals, 1953; Sangvi & Strong, 1958). The following substances, occurring in nature or introduced with feeding, are known to produce liver damage in fish and other animals: zinc chloride, copper sulfate (Calventi, Jakowska & Nigrelli, 1960), carbasone, sulfonamides, antibiotics (Goodman & Gilman, 1955), bentonite (Jakowska & Nigrelli, 1956), radioactive and ionizing substances, and arsenicals (see Hueper, 1953; Wilson, 1954). Their possible role in the etiology of liver diseases in rainbow trout must be considered. In addition, pathological liver changes may be associated with the following well-known diseases of trout: furunculosis, ulcer disease, infectious (viral) pancreatic necrosis, bacterial or viral infections of the kidney, mycosis, helminthiasis, cnidosporidiosis and acute or chronic anemia. Increased pressure in the venous system, resulting from these diseases, may severely affect the liver. The effect on the hepatic cells may result from anoxemia, a condition also associated with acute and chronic anemia

Whatever the cause of the liver lesions in rainbow trout, most of the damage is degenerative, and any hyperplasia seen represents a compensatory response of parenchymal tissue. The pleomorphic changes noted in surrounding and more distant hepatic cells are the result of pressure from the active hyperplasia. It is highly probable that in certain instances the hyperplasia is supervened by neoplasia, especially if the proliferation of the hyperplastic tissue becomes excessive.

Most of the so-called hepatomas in rainbow trout are hyperplasias rather than neoplasias. It has been pointed out by Hartroft (1955) that the differential diagnosis of hyperplasia and neoplasia in the livers of both experimental animals and man has perplexed and confused pathologists for many years; the close resemblance of small neoplastic foci in a cirrhotic liver to foci of hyperplasia irresistibly suggests that the former may arise regularly from the latter.

The sudden and widespread concern about hepatomas in rainbow trout is reminiscent of a similar situation at the turn of the century with regard to thyroid tumors in trout and other salmonids in Europe and the United States. These growths were originally described as

[2] Cystic degeneration of the liver has also been described in rainbow trout in an Italian hatchery by Castelnuovo & Rizzo (1938). It was suggested that this disease is congenital.

adenocarcinomas or carcinomas and, as in the present case of the hepatoma, the disease occurred in epizootic proportions (Plehn, 1902; Pick, 1950; Gaylord & Marsh, 1914). Most of the thyroid tumors were later recognized as simple hyperplasia or goitres (Marine & Lenhart, 1911; Marine, 1914). This interpretation has been substantiated by the fact that since iodine has been regularly added to the water or food, the incidence of thyroid tumors has been reduced to a point where they now are extremely rare, even though a relatively large number of the growths originally described were actually neoplastic. A similar interpretation may prove to be true for the liver tumors of the rainbow trout.

Summary

Microscopical findings in livers of more than 300 hatchery-raised rainbow trout from several states and from rainbow and brown trout from natural streams are described. Most of the livers resembled those of fish kept in captivity and fed artificial diets for prolonged periods. The lesions, when present, were mainly degenerative and characterized by fatty infiltration, glycogen depletion or infiltration, ceroid deposition, hematochromatosis, focal necrosis with lymphocytic infiltration, biliary and portal cirrhosis and other histological and cytological changes. These conditions were associated with compensatory non-nodular and nodular hyperplasia of the liver cells, and it is assumed that under certain circumstances hyperplasia is supervened by neoplasia. Hereditary, nutritional, carcinogenic and other possible etiological factors are discussed.

References

Andervont, H. B.

1950. Studies on the occurrence of spontaneous hepatomas in mice strain C3H and CBA. J. Nat. Cancer Inst., 11: 581-592.

Calventi, Idelisa, Sophie Jakowska & R. F. Nigrelli

1960. Copper sulfate and liver damage in fish (laboratory records).

Casselmen, W. G. B.

1953. Factors influencing the formation of ceroid in the livers of choline deficient rats. Biochem. et Biophys. Acta, II: 445-447.

Castelnuovo, G. & L. Rizzo

1938. Caso di degenerazione cistica del fegato e di altri organi in trota iridea. Boll. di Pesca, Pisciculture e Idrobiologia, 16:1-10.

Cudkowicz, G. & C. Scolari

1955. Un tumore primitivo epatico a diffusione epizootica nella trota iridea di allemamento (*Salmo irideus*). Tumori, XLI (fasc. 5): 524-537.

Daft, F. S.

1954. Experimental differentiation between liver necrosis and liver cirrhosis and some dietary factors affecting their development. Ann. N. Y. Acad. Sci., 57 (6): 623-632. (Conference on Nutritional Factors and Liver Diseases).

Davis, H. S.

1953. "Culture and Diseases of Game Fishes." Univ. California Press. 332 pp.

Drill, A.

1954. Lipotropic effects of vitamin B_{12} and other factors. Ann. N. Y. Acad. Sci., 57 (6): 654-663. (Conference on Nutritional Factors and Liver Diseases).

Duran-Reynals, F.

1953. Virus-induced tumors and the virus theory of cancer. Chapt. 13 in: "Physiopathology of Cancer." Hamburger & Fishman, Ed. Hoeber-Harper, New York, Pp. 298-337.

Elias, H.

1955. Human hepatocarcinoma and the comparative embryology of the vertebrate liver. In: Conference on Experimental Hepatomas. J. Nat. Cancer Inst., 15 (5), suppl. Pp. 1463-1468.

Endicott, K. M. & R. D. Lillie

1944. Ceroid the pigment of dietary cirrhosis of rats. Amer. J. Path., 20: 149-153.

Engle, R. W., D. H. Copeland & W. D. Salmon

1947. Carcinogenic effects associated with diets deficient in choline and related nutrients. Ann. N. Y. Acad. Sci., 49 (1): 49-67.

Faktorovich, K. A.

1956a. (Disease of two year old rainbow trout raised on artificial diets). Voprosy Ikhtiologii, No. 6: 156-173. (In Russian).

1956b. (Regeneration of the liver of rainbow trout). Dokl. Akad. Nauk SSSR, 110 (2): 301-303. (Biol. Abstr., 35 (15) Abst. No. 4191).

1958. (Disturbance in fat metabolism in the liver of rainbow trout raised on artificial diets). Proc. Symposium on Physiology of Fishes, 1956, Acad. Sci. USSR: 237-243. (In Russian).

1960. Uber das Wesen des Stoffes, der Sich in der Leber der Regenbogenforelle bei der sogenannten lipoden Degeneration der Leber Ablagert. Zeitschr. f. Fischerei, 9 (½): 95-99.

Gaschott, O.

1929. Die verlustreichen Leberkrankungen der Forellenmasbetriebe im Früjar 1929. Allg. Fischerei-zeitung, 1929, No. 2: 340-346.

Gaylord, H. R. & M. C. Marsh

1914. Carcinoma of the thyroid in the salmonoid fishes. Bull. Bur. Fish., 32 (1912), doc. no. 790: 363-524.

Goodman, L. S. & A. Gilman

1955. "The Pharmacological Basis of Therapeutics." Macmillan & Co., New York. 1831 pp. (Toxicity effects on liver: carbasone, p. 1218; sulfonamides, p. 1298; antibiotics, p. 1386; copper, p. 1469).

Haddow, A. & Isobel Blake

1933. Neoplasms in fish. A report of six cases with a summary of the literature. J. Path. and Bact., 36: 41-47.

Hartroft, W. S.

1954. The sequence of pathological events in the development of experimental fatty liver and cirrhosis. Ann. N. Y. Acad. Sci., 57 (6): 633-645. (Conference on Nutritional Factors and Liver Diseases).

1955. General histophysiology and histopathology of the liver. J. Nat. Cancer Inst., 15 (5) suppl.: 1463-1468.

Heston, W. E.

1941. Relationship between susceptibility to induced pulmonary tumor and certain known genes in the mouse. J. Nat. Cancer Inst., 2: 127-132.

Heston, W. E. & M. K. Deringer

1947. Relationship between lethal yellow (Ay) gene of mouse and susceptibility to spontaneous pulmonary tumors. J. Nat. Cancer Inst., 7: 463-465.

1949. Relation between hairless gene and the susceptibility to induced pulmonary tumors in mice. J. Nat. Cancer Inst., 10: 119-124.

Hewitt, E. R.

1937. Some recent work on fatty livers in trout. Progr. Fish-culturist, No. 25: 11-15.

Hueper, W. C.

1953. Environmental cancer. Chapt. 26 in: "Physiopathology of Cancer." Hamburger & Fishman, Ed., Hoeber-Harper, New York. Pp. 730-777.

Jakowska, Sophie & R. F. Nigrelli

1956. Effects of diet containing bentonite on the cichlid *Tilapia macrocephala*. Anat. Rec., 125 (3): 655-656.

Kensler, C. J., K. Sugiura, N. F. Young, C. R. Halter & C. P. Rhoads

1941. Partial protection of rats by riboflavin with casein against liver cancer caused by dimethylaminobenzene. Sci., 93: 308-310.

Lee, C. S.

1950. Histochemical studies of the ceroid pigment of rats and mice and its relation to necrosis. J. Nat. Cancer Inst., 11 (2): 339-348.

Mann, H.

1952. Zur Frage der lipoiden Leberdegeneration bei Forelle. Fischereiwelt, 4: 14-15.

Marine, D.

1914. Further observations and experiments on goitre in brook trout. J. Exp. Med., 19: 70-88.

Marine, D. & C. H. Lenhart

1911. Further observations and experiments on so-called thyroid carcinoma of brook trout, and its relation to endemic goitre. J. Exp. Med., 13: 445-475.

McLaren, Barbara A., F. Herman & C. A. Elvehjem

1946. Nutrition of rainbow trout: studies with purified rations. Arch. Biochem., 10: 433-441.

Nigrelli, R. F.

1954. Tumors and other atypical cell growths in temperate freshwater fishes of North America. Trans. Amer. Fish. Soc., 83 (1953): 262-296.

1960. Autopsy and laboratory records, N. Y. Aquarium.

Nigrelli, R. F. & Sophie Jakowska

1955. Spontaneous neoplasms in fishes. IX. Hepatomas in rainbow trout, *Salmo gairdneri*. Proc. Amer. Assoc. Cancer Res., 2 (1): 38.

Pick, L.

1905. Der Schildrüsenkrebs der Salmoniden. Aus dem Laboratorium der L. and Th. Landau'schen Frauenklinik, Berlin. Berliner & Klinische Wschr., 1905 (46/49): 1435-1542.

Pickford, Grace E.

1953. A study of the hypophysectomized male killifish, *Fundulus heteroclitus* (Linn.). Bull. Bingham Oceanogr. Coll., 14 (2): 5-41.

Plehn, Marianne

1902. Bösartiger Kropf (Adeno-Carcinoma der Thyroidea) bei Salmoniden. Allg. Fischerei-Zeitung, no. 7: 117-118.

1909a. Uber einige bei Fischen beobachtete Geschwülste und geschwülstartige Bildungen. Berichte D. K. Bayer. Biol. Versuchs., 2: 55-76.

1909b. Uber die Leber der Salmoniden. Allg. Fischerei-Zeitung, No. 24: 525-527.

1915. Kenntnis der Salmonidenleber im gesunden und kranken Zustand. Zeitschr. f. Fischerei, 17: 1-24.

1924. "Praktikum der Fischkrankheiten." E. Schweizerbart'sche, Stuttgart. Pp. 301-479.

Rucker, R. R., W. T. Yasutake & H. Wolf

1961. Trout hepatoma — a preliminary report. Progr. Fish-culturist, 23 (1): 3-7.

Salmon, W. D. & D. H. Copeland

1954. Liver carcinoma and related lesions in chronic choline deficiency. Ann. N. Y. Acad. Sci., 57 (6): 654-677. (Conference on Nutritional Factors and Liver Diseases).

Salmon, W. D., D. H. Copeland & M. J. Burns

1955. Hepatomas in choline deficiency. J. Nat. Cancer Inst., 15 (5), suppl.: 1549-1554.

Sanghvi, L. D. & L. C. Strong

1958. Effects of selection on chemically induced tumors in mice. Ann. N. Y. Acad. Sci., 71 (6): 839-878. (Conference on Genetic Concept for the Origin of Cancer).

Schäperclaus, W.

1954. "Fischkrankheiten." Akademie, Berlin, 708 pp.

Schwarz, K.

1954. Liver necrosis versus fatty liver and cirrhosis. Ann. N. Y. Acad. Sci., 57 (6): 617-621. (Conference on Nutritional Factors and Liver Diseases).

1954. Factors protecting against dietary necrotic liver degeneration. *ibid*: 878-888.

Scolari, C.

1953. Atti Soc. Ital. Scienze Veter., 7: 599. (See Cudkowicz & Scolari, 1955).

1954. Su di una epizoozia della trote iridee d'allevamento, "la lipoidosi epatica". Clin. Veter., 77 (4): 102-106.

Tannenbaum, A.

1953. Nutrition and Cancer. Chapt. 15 in: "Physiopathology of Cancer." Hamburger & Fishman, Ed. Hoeber-Harper, New York. Pp. 392-437.

Wilson, J. W.

1954. Hepatomas produced by feeding bentonite in the diet. Ann. N. Y. Acad. Sci., 56 (6): 678-683. (Conference on Nutritional Factors and Liver Diseases).

Wood, E. M. & W. T. Yasutake

1956. Ceroid in fish. Amer. J. Path., 32: 591-603

Woolley, G. W.

1953. Genetics of neoplastic diseases. Chapt. 11 in: "Physiopathology of Cancer." Hamburger & Fishman. Ed. Hoeber-Harper, New York. Pp. 171-224.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. Female rainbow trout (*Salmo gairdneri* Richardson) showing discolored lesions on the liver. Reduced 2.5 ×.

Fig. 2. Section through an encapsulated tumor mass of rainbow trout from a Pennsylvania hatchery. 5 ×.

Plate II

Fig. 3. Fatty infiltration in an otherwise normal liver of a rainbow trout. Hematoxylin-eosin. 500 ×.

Fig. 4. Extensive ceroid deposition of liver. Acid-fast stain. 250 ×.

Fig. 5. Accumulation of siderin in areas surrounding biliary duct. Hematoxylin-eosin. 1000 ×.

Plate III

Fig. 6. "Normal" lobule of liver of rainbow trout with two-cell-thick muralia radiating from a central vein. Note necrotic areas adjacent to the lobule. Hematoxylin-eosin. 250 ×.

Fig. 7. Typical lymphocytic infiltration in a necrotic area. Hematoxylin-eosin. 500 ×.

Fig. 8. Section of otherwise "normal" liver showing portal cirrhosis and a small necrotic area. Hematoxylin-eosin. 250 ×.

Fig. 9. Biliary cirrhosis and disorganization of adjacent hepatic elements. Masson's stain. 400 ×.

Plate IV

Fig. 10. Focal necrosis and localized hyperplasia of liver cells from rainbow trout. Hematoxylin-eosin. 250 ×.

Fig. 11. Cluster of acidophilic cells with eccentric nuclei. Some cells contain ceroid. The nature and origin of these cells are unknown. Hematoxylin-eosin. 500 ×.

Fig. 12. Diffuse regenerative hyperplasia associated with extensive degeneration of the liver. Hematoxylin-eosin. 400 ×.

Plate V

Fig. 13. Adenomatoid appearance of hepatic tissue associated with hyperplastic foci in more distantly located areas of the same section. Hematoxylin-eosin. 400 ×.

Fig. 14. Details of adenomatous liver. Note large ceroid globule. Masson's stain. 2000 ×.

Fig. 15. Cord-like arrangement of hepatocellular tumor. Hematoxylin-eosin. 600 ×.

Plate VI

Fig. 16. Cholangiomatous changes in hepatoma of rainbow trout. Hematoxylin-eosin. 500 ×.

Fig. 17. Extensive cirrhosis seen in hepatoma of Pennsylvania rainbow trout. Hematoxylin-eosin. 250 ×.